09/915,780

WEST

Freeform Search

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Term: L2 and allele specific

Display: 10 **Documents in Display Format:** - **Starting with Number** 1

Generate: ○ Hit List ◉ Hit Count ○ Side by Side ○ Image

Search | Clear | Help | Logout | Interrupt

Main Menu | Show S Numbers | Edit S Numbers | Preferences | Cases

Search History

DATE: Saturday, November 30, 2002 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L3	L2 and allele specific	8	L3
L2	primer$1 near5 non complementary near5 end	33	L2
DB=DWPI,USPT,EPAB,JPAB; PLUR=YES; OP=ADJ			
L1	primer$1 near5 non complementary near5 5 end41	0	L1

END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 8 of 8 returned.

☐ 1. 6468791 . 16 Aug 99; 22 Oct 02. Chromosone 1 gene and gene products related to Alzheimer's Disease. Tanzi; Rudolph E., et al. 435/325; 435/320.1 435/69.1 536/23.1 536/23.5 536/24.31. C12N015/00 C12N015/63.

☐ 2. 6379889 . 04 Nov 99; 30 Apr 02. Multiplexing methods for identifying nucleic acids using denaturing liquid chromatography. Apffel, Jr.; James A., et al. 435/6; 436/172 436/800 436/805 436/94 536/23.1 536/24.3 536/25.3. C12Q001/68 G01N033/00 C07H021/02.

☐ 3. 6335184 . 11 Jan 99; 01 Jan 02. Linked linear amplification of nucleic acids. Reyes; Antonio Arevalo, et al. 435/91.2; 435/6. C12P019/34.

☐ 4. 6248555 . 30 Aug 96; 19 Jun 01. Genetic alterations related to familial alzheimer's disease. Tanzi; Rudolph, et al. 435/69.1; 435/252.3 435/320.1 435/325 536/23.1 536/23.5. C12N015/12 C12N015/63 C12N015/85.

☐ 5. 6171785 . 06 Jun 95; 09 Jan 01. Methods and devices for hemogeneous nucleic acid amplification and detector. Higuchi; Russell G.. 435/6; 435/91.2 436/63 436/94. C12Q001/68 C12P019/34 C12N015/10.

☐ 6. 6090620 . 27 Dec 96; 18 Jul 00. Genes and gene products related to Werner's syndrome. Fu; Ying-Hui, et al. 435/325; 435/320.1 435/455 435/69.1 536/23.5 536/24.31 800/13. C12N005/00 C12N015/00 C12N015/63 C12N015/09.

☑ 7. 6027923 . 02 Apr 97; 22 Feb 00. Linked linear amplification of nucleic acids. Wallace; Robert Bruce. 435/91.2; 435/6. C12P019/34.

☐ 8. 5994056 . 02 May 91; 30 Nov 99. Homogeneous methods for nucleic acid amplification and detection. Higuchi; Russell G.. 435/6; 435/810 435/91.2 436/63 436/94. C12P001/48 C12P001/68 C12N015/10.

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Term	Documents
ALLELE.DWPI,EPAB,JPAB,USPT.	7925
ALLELES.DWPI,EPAB,JPAB,USPT.	7608
SPECIFIC.DWPI,EPAB,JPAB,USPT.	2010710
SPECIFICS.DWPI,EPAB,JPAB,USPT.	11746
(2 AND (ALLELE ADJ SPECIFIC)).USPT,JPAB,EPAB,DWPI.	8
(L2 AND ALLELE SPECIFIC).USPT,JPAB,EPAB,DWPI.	8

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End of Result Set

☐ Generate Collection

L3: Entry 8 of 8 File: USPT Nov 30, 1999

DOCUMENT-IDENTIFIER: US 5994056 A
TITLE: Homogeneous methods for nucleic acid amplification and detection

Brief Summary Text (10):
Other means of detection include the use of fragment length polymorphism hybridization, allele-specific oligonucleotide (ASO) probes (Saiki et al., 1986, Nature 324:163), or direct sequencing via the dideoxy method using amplified DNA rather than cloned DNA. The fragment length polymorphism method detects insertions and deletions between PCR primers resulting in PCR products of different lengths, detectable by sizing. ASO methods are useful for detecting allelic sequence variations. In an example of ASO hybridization, the amplified DNA is fixed to a nylon filter (by, for example, UV irradiation) in a series of "dot blots," then allowed to hybridize with an oligonucleotide probe under stringent conditions. This method is also described in copending U.S. Ser. No. 347,495, filed May 4, 1989, which is incorporated herein by reference. The probe may be labeled with, for example, horseradish peroxidase (HRP) and detected by the presence of a blue precipitate following treatment with suitable oxidation reagents.

Detailed Description Text (50):
Preferably, the DNA binding agent is storage stable and can be included as a component in a PCR reagent buffer. Thus, the invention provides novel reagents suitable for commercialization in a kit format. Such a reagent may contain a solution of the DNA binding agent in a kit for detecting nucleic acids by PCR. Alteratively, the reagent might contain a DNA binding agent, as well as other PCR buffer components such as Tris-HCl, KCl, and MgCl.sub.2, each in appropriate concentrations for carrying out PCR. In one embodiment, a kit includes a buffer comprising ethidium bromide at a suitable concentration to provide, in an amplification reaction, a final ethidium bromide concentration in the range of 0.15 .mu.M to 20.3 .mu.M. The buffer may additionally contain any or all of the following reagents: Tris-HCl, pH 8.0-8.3; KCl, and MgCl.sub.2 each in appropriate concentrations for PCR amplification. Kits for detecting amplified nucleic acids are also envisioned as including any of the following: an agent for polymerization, dNTPs, appropriate primers, and a positive control template. The '202 patent describes methods for preparing and using primers for PCR which have non-complementary sequences added to the 5' end. These "tails" are useful for engineering particular restriction sites or other purposes, because during PCR, the non-complementary tail sequence is incorporated into the double-stranded PCR product. Particular tail sequences provide binding targets for specific dyes. For example, Hoechst 33258 (Searle and Embrey, 1990, Nuc. Acids Res. 18:3753-3762) preferentially binds A-T base pairs. A PCR primer synthesized with a long A-T rich 5' tail provides a relatively A-T rich PCR product in comparison with genomic DNA. Using Hoechst 33358 the A-T rich PCR product has increased fluorescence relative to genomic DNA and, consequently, is useful for increasing signal strength in the presence of genomic DNA.

Detailed Description Text (95):
This example demonstrates the suitability of the homogeneous assay not only for detecting a single copy gene among total human genomic DNA, but for discriminating among two alleles of that single copy gene present in the sample that differ by a single nucleotide. (Methods for allele specific detection are described in detail in European Patent Publication No. 237,362, which is incorporated herein by reference).

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Freeform Search

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Term: L5 and mismatch$2

Display: 10 **Documents in Display Format:** - **Starting with Number** 1

Generate: ○ Hit List ◉ Hit Count ○ Side by Side ○ Image

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Main Menu | Show S Numbers | Edit S Numbers | Preferences | Cases

Search History

DATE: Saturday, November 30, 2002 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
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L7	L6 and (5 end near5 binding pair)	0	L7
L6	L5 and mismatch$2	3	L6
L5	l1 and (3 end near5 penultimate)	3	L5
L4	l2 and (binding or label$1)	1	L4
L3	L2 and binding pair	0	L3
L2	L1 and (3 end near5 penultimate near5 mismatch)	1	L2
L1	allele specific near5 (primer$1 or probe$1)	1106	L1

END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 3 of 3 returned.

☐ 1. 6312892 . 10 Jul 97; 06 Nov 01. High fidelity detection of nucleic acid differences by ligase detection reaction. Barany; Francis, et al. 435/6; 435/91.1 435/91.2 435/91.5 435/91.51. C12Q001/68 C12P019/34.

☑ 2. 6235480 . 21 Jul 99; 22 May 01. Detection of nucleic acid hybrids. Shultz; John William, et al. 435/6; 435/91.2 435/91.5 436/173 436/501. C12Q001/68 C12P019/34 G01N024/00 C07H019/04.

☑ 3. 6156503 . 03 Mar 97; 05 Dec 00. Diagnosing asthma patients predisposed to adverse .beta.-agonist reactions. Drazen; Jeffrey M., et al. 435/6; 435/91.2 536/23.5 536/24.31 536/24.33. C12Q001/68 C07H021/04.

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Term	Documents
MISMATCH$2	0
MISMATCH.DWPI,EPAB,JPAB,USPT.	37325
MISMATCHCS.DWPI,EPAB,JPAB,USPT.	2
MISMATCHE.DWPI,EPAB,JPAB,USPT.	2
MISMATCHED.DWPI,EPAB,JPAB,USPT.	8935
MISMATCHER.DWPI,EPAB,JPAB,USPT.	1
MISMATCHES.DWPI,EPAB,JPAB,USPT.	9661
MISMATCHN:.DWPI,EPAB,JPAB,USPT.	1
MISMATCHS.DWPI,EPAB,JPAB,USPT.	6
MISMATCHTO.DWPI,EPAB,JPAB,USPT.	1
MISMATCH0.DWPI,EPAB,JPAB,USPT.	2
(L5 AND MISMATCH$2).USPT,JPAB,EPAB,DWPI.	3

There are more results than shown above. Click here to view the entire set.

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L6: Entry 1 of 2 File: USPT May 7, 2002

DOCUMENT-IDENTIFIER: US 6383742 B1
TITLE: Three dimensional arrays for detection or quantification of nucleic acid species

Detailed Description Text (103):
Other labels include ligands which can serve as specific binding members to a labeled antibody, chemiluminescers, enzymes, antibodies which can serve as a specific binding pair member for a labeled ligand, and the like. A wide variety of labels have been employed in immunoassays which can readily be employed. Still other labels include antigens, groups with specific reactivity, and electrochemically detectable moeities.

Detailed Description Text (125):
The use of CovaLink NH strips for covalent binding of DNA molecules at the 5'-end has been described (Rasmussen et al., 1991). In this technology, a phosphoramidate bond is employed (Chu et al., 1983). This is beneficial as immobilization using only a single covalent bond is preferred. The phosphoramidate bond joins the DNA to the CovaLink NH secondary amino groups that are positioned at the end of spacer arms covalently grafted onto the polystyrene surface through a 2 nm long spacer arm. To link an oligonucleotide to CovaLink NH via an phosphoramidate bond, the oligonucleotide terminus must have a 5'-end phosphate group. It is, perhaps, even possible for biotin to be covalently bound to CovaLink and then streptavidin used to bind the probes.

Other Reference Publication (87):
Saiki, R.K., Bugawan, T.L., Horn, G.T., Mullis, K.B., and Erlich, H.A., (1986), "Analysis of Enzymatically Amplified .beta.-Globin and HLA-DQ.alpha. DNA with Allele-Specific Oligonucleotide Probes," Letters to Nature, 324, 163-166.

End of Result Set

☐ Generate Collection

L6: Entry 3 of 3 File: USPT Dec 5, 2000

DOCUMENT-IDENTIFIER: US 6156503 A
TITLE: Diagnosing asthma patients predisposed to adverse .beta.-agonist reactions

Detailed Description Text (45):
ARMS ASSAY: The primers used to detect the .beta..sub.2 -adrenergic receptor gene polymorphism (corresponding to an A.fwdarw.G substitution at nucleotide 1633 of SEQ ID NO:2) (A.fwdarw.G) that gives rise to the Arg 16.fwdarw.Gly amino acid change in the protein were: Wild-type forward primer A1 (5'-GCCTCTTGCTGGCACCCAA-AA-3' [SEQ ID NO:3]) corresponding to nucleotides 1612-1633, except the penultimate base at the 3' end (underlined) was changed from T to A, polymorphism-specific forward primer A2 (5'-GCCTTCTTGCTGGCACCCAAAG-3' [SEQ ID NO:4], differs from the wild-type primer at the last nucleotide at 3' end (shown in bold), reverse primer Rev (5'-AGGATAACCTCATCCGTAAGG-3' [SEQ ID NO:5]) corresponding to nucleotides 2483-2503 on the complementary strand.

Detailed Description Text (46):
The primers used to detect the .beta..sub.2 -adrenergic receptor gene polymorphism (corresponding to a C.fwdarw.G substitution at nucleotide 1666 of SEQ ID NO:2) that gives rise to the Gln27.fwdarw.Glu amino acid change in the protein were: Wild-type forward primer B1 (5'-CCGGACCACGACGTCACGCAAC-3' [SEQ ID NO:6] corresponding to nucleotides 1645-1666, except the penultimate base at the 3' end (underlined) was changed from G to A, polymorphism-specific forward primer B2 (5'-CCGGACCACGACGTCACGCAAG-3' [SEQ ID NO:7]), differs from the wild-type primer at the last nucleotide at 3' end (shown in bold), and reverse primer Rev.

Detailed Description Text (47):
Amplification by PCR of the genomic DNA of each sample included two reactions for each assay separately: one with wild type primers (A1 and REV) and the other with polymorphic (A2 and Rev) allele-specific primer set for polymorphism detection at nucleotide 16633 and wild type primers (B1 and Rev) and the polymorphic allele-specific primer set (B2 and Rev) for polymorphism detection at nucleotide 1666. Both polymorphism detection assays included human .beta.-globin primer sets as positive controls in the PCR reaction mix. The primers for .beta.-globin were: Forward primer BG1 (5'-GCTGTCATCACTTAGACCTC-3' [SEQ ID NO:8] corresponding to nucleotides 43-62 (Genbank accession no. 148217), reverse primer BG2 (5'-CAGACGAATGATTGCATCAG-3' [SEQ ID NO: 9]) corresponding to nucleotides 766-785 on the complementary strand (Genbank accession no. L48217).

Detailed Description Text (65):
GENERALLY: One preferred method for identifying .beta..sub.2 -adrenergic receptor gene polymorphisms in order to practice the present invention is to perform polymerase chain reactions (PCR) using primers whose 3'-most nucleotide is mismatched with respect to either the Arg 16 allele or the Gly 16 allele (see Newton et al., Nuc. Acids. Res. 17:2503, 1989, incorporated herein by reference; see also Example 2). The PCR reaction conditions are then adjusted so that product band is only produced when the primer and template are matched.

Detailed Description Text (66):
Useful PCR primers and conditions for detection of the Arg 16 and Gly 16 .beta.2-adrenergic receptor gene alleles have been described (Turki et al., J. Clin. Invest. 95:1635, 1995, incorporated herein by reference; see also Example 2). As described in that article, allele-specific PCR is based on the premise that, under the appropriate conditions, a match between template and primer at the most 3' nucleotide is necessary for the generation of a PCR product (i.e., mismatches result in no

product). Allele-specific PCR reactions can be performed, for example, as follows:

Detailed Description Text (77):
As will be apparent to those of ordinary skill in the art, reagents useful in the practice of the present inventive methods can usefully be collected together in kits. For example, primer sets for allele-specific polymerase chain reaction studies can be provided together in a single container.

Detailed Description Text (78):
As described above in Example 3, .beta..sub.2 -adrenergic receptor gene alleles can be distinguished from one another through use of primers whose 3'-most nucleotides hybridize with one allele but are mismatched with respect to others. Examples 2 and 3 describe particular useful primer sets, but those of ordinary skill in the art will readily recognize that variations in precise primer sequence can be made without departing from the spirit or scope of the present invention, so long as one primer set produces an amplification product from one .beta..sub.2 -adrenergic receptor gene allele (e.g., the allele encoding the Arg16 variant), and a different primer set produces an amplification product from another allele (e.g., the allele encoding the Gly16 variant). Preferred allele-specific PCR kits also include other PCR reagents, such as buffer, salt solutions, dNTPs, control DNA including the Arg16 .beta..sub.2 -adrenergic receptor gene allele, control DNA including the Gly16 .beta..sub.2 -adrenergic receptor gene allele, and/or DNA polymerase. Preferably, the DNA polymerase is thermal-stable. Such kits may optionally include instructions for use.

Detailed Description Text (80):
Primer-containing kits may also be desirably prepared that do not contain allele-specific primer sets, but rather contain only a single set of primers, which primers amplify a region of the .beta..sub.2 -adrenergic receptor gene that encodes residue 16. Preferred such kits also include PCR reagents and/or sequencing reagents. Preferably, dideoxy sequencing reagents are employed (see Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989, Chapter 13, incorporated herein by reference). Preferred dideoxy sequencing reagents include, for example, a sequencing primer (that hybridizes either to the .beta..sub.2 -adrenergic receptor gene amplification product or to a vector into which the product may be cloned), dNTPs, ddNTPs, buffers, salts, and/or instructions. In preferred embodiments, the dNTPs are provided either singly or in mixtures that are sets of three dNTPs. Preferred kits may also (or alternatively) include detection reagents, such as, for example, radioactive or fluorescent. Particularly preferred kits are designed genetic analyzers and include fluorescently-tagged primers.

End of Result Set

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L4: Entry 1 of 1 File: USPT Nov 6, 2001

DOCUMENT-IDENTIFIER: US 6312892 B1
TITLE: High fidelity detection of nucleic acid differences by ligase detection reaction

Brief Summary Text (11):
Another scheme for multiplex detection of nucleic acid sequence differences is disclosed in U.S. Pat. No. 5,470,705 to Grossman et. al. where sequence-specific probes, having a detectable label and a distinctive ratio of charge/translational frictional drag, can be hybridized to a target and ligated together. This technique was used in Grossman, et. al., "High-density Multiplex Detection of Nucleic Acid Sequences: Oligonucleotide Ligation Assay and Sequence-coded Separation," Nucl. Acids Res. 22(21): 4527-34 (1994) for the large scale multiplex analysis of the cystic fibrosis transmembrane regulator gene.

Brief Summary Text (17):
Ligation of allele-specific probes generally has used solid-phase capture (U. Landegren et al., Science, 241:1077-1080 (1988); Nickerson et al., Proc. Natl. Acad. Sci. USA, 87:8923-8927 (1990)) or size-dependent separation (D. Y. Wu, et al., Genomics, 4:560-569 (1989) and F. Barany, Proc. Natl. Acad. Sci., 88:189-193 (1991)) to resolve the allelic signals, the latter method being limited in multiplex scale by the narrow size range of ligation probes. Further, in a multiplex format, the ligase detection reaction alone cannot make enough product to detect and quantify small amounts of target sequences. The gap ligase chain reaction process requires an additional step--polymerase extension. The use of probes with distinctive ratios of charge/translational frictional drag for a more complex multiplex process will either require longer electrophoresis times or the use of an alternate form of detection.

Brief Summary Text (35):
Other procedures have been developed to detect minority nucleotide sequences. Lu, et. al., "Quanititative Aspects of the Mutant Analysis by PCR and Restriction Enzyme Cleavage (MAPREC)" PCR Methods and Appl. 3: 176-80 (1993) detects virus revertants by PCR and restriction enzyme cleavage. The disadvantages of MAPREC include the requirement for electrophoretical separation to distinguish mutant from normal DNA, limited applicability to sites that may be converted into a restriction site, the requirement for additional analysis to determine the nature of the mutation, and difficulty in quantifying mutant DNA in a high background of normal DNA. In Kuppuswamy, et. al., "Single Nucleotide Primer Extension to Detect Genetic Diseases: Experimental Application to Hemophilia G (Factor IX) and Cystic Fibrosis Genes," Proc. Natl. Acad. Sci. USA 88: 1143-47 (1991), a PCR process is carried out using 2 reaction mixtures for each fragment to be amplified with one mixture containing a primer and a labeled nucleotide corresponding to the normal coding sequence, while the other mixture contains a primer and a labeled nucleotide corresponding to the mutant sequence. The disadvantages of such mini sequencing (i.e. SNuPe) are that the mutations must be known, it is not possible to multiplex closely clustered sites due to interference of overlapping primers, it is not possible to detect single base or small insertions and deletions in small repeat sequences, and four separate reactions are required. A mutagenically separated PCR process is disclosed in Rust, et. al., "Mutagenically Separated PCR (MS-PCR): a Highly Specific One Step Procedure for easy Mutation Detection" Nucl. Acids Res. 21(16): 3623-29 (1993) to distinguish normal and mutant alleles, using different length allele-specific primers. The disadvantages of MS-PCR include possibly providing false positives due to polymerase extension off normal template, requiring electrophoretical separation of products to distinguish from primer dimers, the inability to multiplex closely-clustered sites due to interference of overlapping primers, the inability to detect single base or small

insertions and deletions in small repeat sequences, and not being ideally suited for quantification of mutant DNA in high background of normal DNA. In Suzuki, et. al., "Detection of ras Gene Mutations in Human Lung Cancers by Single-Strand Conformation Polymorphism Analysis of Polymerase Chain Reaction Products," Oncogene 5: 1037-43 (1990), mutations are detected in a process having a PCR phase followed by phase involving single strand conformation polymorphism ("SSCP") of the amplified DNA fragments. The disadvantages of SSCP include the requirement for electrophoretical separation to distinghish mutant conformer from normal conformer, the failure to detect 30% of possible mutations, the requirement for additional analysis to determine the nature of the mutation, and the inability to distinguish mutant from silent polymorphisms.

Drawing Description Text (3):
FIG. 1 is a flow diagram depicting a PCR/LDR process for detection of cancer-associated mutations by electrophoresis or capture on an addressable array where wild-type allele-specific oligonucleotide probes are excluded from the LDR phase to avoid overwhelming signal from minority mutant target and no marker is added to the LDR phase.

Drawing Description Text (4):
FIG. 2 is a flow diagram depicting a PCRILDR process for detection of cancer-associated mutations by electrophoresis or capture on an addressable array where wild-type allele-specific oligonucleotide probes are excluded from the LDR phase to avoid overwhelming signal from minority mutant target and a marker is added to the LDR phase.

Drawing Description Text (5):
FIG. 3 is a flow diagram depicting a PCR/LDR process for detection of cancer-associated mutations by electrophoresis or capture on an addressable array where wild-type allele-specific oligonucleotide probes are utilized in the LDR phase at low levels and/or are modified to yield less ligation product corresponding to the majority target. This prevents the signal from minority mutant target from being overwhelmed.

Drawing Description Text (39):
FIGS. 30A-C show a scheme for PCR/LDR detection of mutations in codons 12, 13, and 61 of K-ras. At the top of the drawing (FIG. 30A) is a schematic representation of the chromosomal DNA containing the K-ras gene. Exons are shaded and the position of codons 12, 13, and 61 shown. Exon-specific primers are used to amplify selectively K-ras DNA flanking these three codons. The middle (FIG. 30B) and bottom (FIG. 30C) of the diagram gives a schematic representation of primer design for LDR detection of all possible amino acid changes in codons 12, 13, and 61. For example, codon 12 (GGT) may mutate to GAT, GCT, or GTT. Allele-specific LDR oligonucleotide probes contain the discriminating base on the 3' end and a fluorescent label on the 5' end. Common oligonucleotides are phosphorylated on the 5' end and contain a poly-A tail and blocking group on the 3' end. Different mutations are distinguished by separating the products on a polyacrylamide gel. Note that LDR oligonucleotide probes used for detecting mutations at codon 12 may interfere with hybridization of oligonucleotide probes used to detect mutations at codon 13. It will be necessary to determine experimentally if these probes can correctly identify mutant signal in the presence of the other LDR probes.

Detailed Description Text (6):
In effecting detection/quantification, there are 3 techniques of practicing the PCR/LDR process in accordance with the present invention with each being practiced using either of two formats. More particularly, the LDR phase can be carried out by (1) excluding wild-type allele-specific oligonucleotide probes from the LDR phase to avoid overwhelming signal from the minority mutant target and adding no marker, (2) excluding wild-type allele-specific oligonucleotide probes from the LDR phase but adding a marker to that phase, and (3) utilizing wild-type allele-specific oligonucleotide probes in the LDR phase at low levels and/or modified forms of those probes to yield less ligation product corresponding to the majority target which prevents signal from the minority mutant target from being overwhelmed. One detection format alternative involves use of capillary electrophoresis or gel electrophoresis and a fluorescent quantification procedure. Alternatively, detection can be carried

out by capture on an array of capture oligonucleotide addresses and fluorescent quantification. These alternatives are explained more fully with reference to FIGS. 1-9.

Detailed Description Text (7):
FIG. 1 depicts the detection of cancer-associated mutations where wild-type allele-specific oligonucleotide probes are excluded from the LDR phase to avoid overwhelming signal from minority mutant target and no marker is added to the LDR phase. In step 1, after DNA sample preparation, multiple exons are subjected to PCR amplification using Taq polymerase under hot start conditions with target-specific oligonucleotide primers. The extension products produced during PCR are then diluted 1/20 during step 2. In step 3, the extension products are mixed with oligonucleotide probes containing allele-specific portions and common portions and the LDR phase of the process is initiated by addition of Taq ligase under hot start conditions. During LDR, oligonucleotide probes ligate to their adjacent oligonucleotide only in the presence of target sequence which gives perfect complementarity at the ligation junction. Absence of wild-type allele-specific oligonucleotide probes, and consequently absence of wild-type specific ligation product prevents the ligation detection reaction signal generated by minority mutant target from being overwhelmed.

Detailed Description Text (9):
FIG. 2 depicts the detection of cancer-associated mutations where wild-type allele-specific oligonucleotide probes are excluded from the LDR phase to avoid overwhelming signal from minority mutant target and a marker is added to the LDR phase. In step 1, after DNA sample preparation, multiple exons are subjected to PCR amplification using Taq polymerase under hot start conditions with target-specific oligonucleotide primers. Fluorescent quantification of PCR products can be achieved using capillary or gel electrophoresis in step 2. In step 3, the products are spiked with a 1/100 dilution of marker DNA (for each of the fragments). This DNA is homologous to wild type DNA, except it contains a mutation which is not observed in cancer cells, but which may be readily detected with the appropriate LDR probes. In step 4, the mixed DNA products from the PCR phase are then diluted 20-fold into fresh LDR buffer containing LDR oligonucleotide probes containing allele-specific portions and common portions. Step 5 involves the LDR phase of the process which is initiated by addition of Taq ligase under hot start conditions. During LDR, oligonucleotide probes ligate to their adjacent oligonucleotide probes only in the presence of target sequence which gives perfect complementarity at the junction site.

Detailed Description Text (11):
FIG. 3 depicts the detection of additional cancer-associated mutations where additional wild-type allele-specific oligonucleotide probes are utilized in the LDR phase at low levels and/or are modified to yield less ligation product corresponding to the majority target. In step 1, after DNA sample preparation, multiple exons are subjected to PCR amplification using Taq polymerase under hot start conditions with target-specific oligonucleotide primers. The extension products produced during PCR are then diluted 1/20 during step 2. In step 3, the extension products are mixed with oligonucleotide probes containing allele-specific portions and common portions and the LDR phase of the process is initiated by addition of Taq ligase under hot start conditions. During LDR, oligonucleotide probes ligate to their adjacent oligonucleotide only in the presence of target sequence which gives perfect complementarity at the ligation junction. Due to the concentration and/or modification of the wild-type allele-specific oligonucleotide probes, the level of ligation product generated with these probes is comparable to the amount of ligation product generated from the minority target nucleotide sequences.

Detailed Description Text (12):
The products can be detected by either of two formats. In the format of step 4a, products are separated by capillary or gel electrophoresis, and fluorescent signal quantified. By way of example, consider the low level and/or modified wild-type allele-specific oligonucleotide probes ligating on a given amount of majority target nucleotide sequence (i.e. 1 picomole) generating the same amount of ligation product as generated from a given minority target sequence (using minority allele-specific oligonucleotide probes) present as a 100-fold dilution (i.e. 10 femtomoles) in the same amount (i.e. 1 picomole) of majority target nucleotide sequence. The ratio of mutant peaks to wild-type peaks gives the approximate amount of minority target

(cancer-associated mutations) present in the original sample divided by 100. In the format of step 4b, products are detected by specific hybridization to complementary sequences on an addressable array. Amount of minority product is quantified as described above.

Detailed Description Text (17):
The oligonucleotide probe sets, as noted above, have a reporter label suitable for detection. Useful labels include chromophores, fluorescent moieties, enzymes, antigens, heavy metals, magnetic probes, dyes, phosphorescent groups, radioactive materials, chemiluminescent moieties, and electrochemical detecting moieties.

Detailed Description Text (21):
Another quantification method, in accordance with the present invention, relates to an internal standard. Here, a known amount of one or more marker target nucleotide sequences is added to the sample. In addition, one or a plurality of marker-specific oligonucleotide probe sets are added along with the ligase, the previously-discussed oligonucleotide probe sets, and the sample to a mixture. The marker-specific oligonucleotide probe sets have (1) a first oligonucleotide probe with a target-specific portion complementary to the marker target nucleotide sequence, and (2) a second oligonucleotide probe with a target-specific portion complementary to the marker target nucleotide sequence and a detectable reporter label. The oligonucleotide probes in a particular marker-specific oligonucleotide set are suitable for ligation together when hybridized adjacent to one another on a corresponding marker target nucleotide sequence. However, there is a mismatch which interferes with such ligation when hybridized to any other nucleotide sequence present in the sample or added marker sequences. The presence of ligation product sequences is identified by detection of reporter labels. The amount of target nucleotide sequences in the sample is then determined by comparing the amount of ligation product sequence generated from known amounts of marker target nucleotide sequences with the amount of other ligation product sequences.

Detailed Description Text (23):
Another quantification method, in accordance with the present invention, involves analysis of a sample containing two or more of a plurality of target nucleotide sequences with a plurality of sequence differences, where one or more target nucleotide sequences is in excess (majority) over other minority target nucleotide sequences. Here, in addition to the allele-specific oligonucleotide probes for the minority target nucleotide sequences, modified wild-type allele-specific oligonucleotide probes are also utilized in the LDR phase at low levels and/or are modified to yield less ligation product corresponding to the majority target. The presence of both minority target specific ligation products and majority target specific ligation products is identified by detection of reporter labels. The amount of minority target nucleotide sequences in the sample is determined by comparing the amount of low yield ligation product sequences generated from the majority target nucleotide sequences with the amount of other ligation products.

Detailed Description Text (40):
FIG. 4 is a schematic diagram depicting the PCR/LDR process of FIG. 1 using electrophoresis to separate ligation products. More particularly, this diagram relates to detection of codon 12 of the K-ras gene which has a GGT sequence that codes for glycine ("Gly"). A small percentage of the cells contain the G to A mutation in GAT, which codes for aspartic acid ("Asp"). As illustrated, this process involves an initial PCR amplification in step 1, an LDR procedure in step 2, and a separation of fluorescent products followed by quantification in step 3. Alternatively, step 3 can involve ethidium bromide staining or running an additional LDR reaction on diluted product using normal oligonucleotide probes (See FIGS. 23 and 29 infra). The LDR probes for wild-type (i.e. normal) sequences are missing from the reaction. If the normal LDR probes (with the discriminating base being G) were included, they would ligate to the common probes and overwhelm any signal coming from the minority mutant target. Instead, as shown in FIG. 4, the existence of a 44 base ligation product sequence with fluorescent label F1 coupled to a single nucleotide (designated N.sub.1) and the A.sub.n tail indicates the presence of the aspartic acid encoding mutant. This ligation product sequence has the same F1 label as that formed by the existence of the arginine and valine encoded mutations. However, these sequences are distinguishable by virtue of their different lengths due to different length tails and different numbers

of nucleotides N coupling the label to the remainder of the ligation product sequence. More particularly, the presence of an F1 labelled, 48 base ligation product sequence suggests the presence of the arginine encoding codon, while the presence of an F1 labelled, 46 base ligation product sequence indicates the presence of the valine encoding codon. These ligation product sequences are distinguished by size with the longer products having a lower electrophoretic mobility. The F2 labelled ligation products are similarly distinguished by their length which varies as a result of the different length tails and the number of nucleotides N coupling the label to the remainder of the ligation product sequence. More particularly, the 49 base ligation product sequence (due to 2 nucleotides N coupling the label and the A.sub.n+4 tail) indicates the presence of the cysteine encoding codon, the 47 base ligation product sequence (due to no nucleotides N coupling the label and the A.sub.n+4 tail) indicates the presence of the serine encoding codon, and the 45 base ligation product sequence (due to 2 nucleotides N coupling the label and the A.sub.n tail) indicates the presence of the alanine encoding codon.

Detailed Description Text (41):
FIG. 5 is a schematic diagram depicting the PCR/LDR process of FIG. 2 using electrophoresis to separate ligation products. More particularly, this diagram relates to detection of codon 12 of the K-ras gene which has a GGT sequence that codes for glycine ("Gly"). A small percentage of the cells contain the G to A mutation in GAT, which codes for aspartic acid ("Asp"). As illustrated, this process involves an initial PCR amplification in step 1, an LDR procedure in step 2, and a separation of fluorescent products followed by quantification in step 3. After amplification, the PCR products are quantified. A marker template is added prior to the LDR phase where both allele-specific and marker-specific oligonucleotide probes are utilized. The LDR probes for wild-type (i.e. normal) sequences are missing from the reaction. If the normal LDR probes (with the discriminating base being G) were included, they would ligate to the common probes and overwhelm any signal coming from the minority mutant target. Instead, as shown in FIG. 5, the existence of a 44 base ligation product sequence with fluorescent label F1 coupled to a single nucleotide (designated N.sub.1) and the A.sub.n tail indicates the presence of the aspartic acid encoding mutant. This ligation product sequence has the same F1 label as that formed by the existence of the arginine and valine encoded mutations. However, these sequences are distinguishable by virtue of their different lengths due to different length tails and different numbers of nucleotides N coupling the label to the remainder of the ligation product sequence. More particularly, the presence of an F1 labelled, 48 base ligation product sequence suggests the presence of the arginine encoding codon, while the presence of an F1 labelled, 46 base ligation product sequence indicates the presence of the valine encoding codon. These ligation product sequences are distinguished by size with the longer products having a lower electrophoretic mobility. The F2 labelled ligation products are similarly distinguished by their length which varies as a result of the different length tails and the number of nucleotides N coupling the label to the remainder of the ligation product sequence. More particularly, the 49 base ligation product sequence (due to 2 nucleotides N coupling the label and the A.sub.n+4 tail) indicates the presence of the cysteine encoding codon, the 47 base ligation product sequence (due to no nucleotides N coupling the label and the A.sub.n+4 tail) indicates the presence of the serine encoding codon, and the 45 base ligation product sequence (due to 2 nucleotides N coupling the label and the A.sub.n tail) indicates the presence of the alanine encoding codon. The ligation product formed by the marker-specific oligonucleotide probe is 43 bases and has the F.sub.2 label (due to 0 nucleotides N coupling the label and the A.sub.n tail). As discussed above, the amount of minority target nucleotide sequences in the sample is determined by comparing the amount of ligation product sequence generated from known amounts of marker target nucleotide sequences with the amount of other ligation product sequences.

Detailed Description Text (42):
FIG. 6 is a schematic diagram depicting the PCR/LDR process of FIG. 3 using electrophoresis to separate ligation products. More particularly, this diagram relates to detection of codon 12 of the K-ras gene which has a GGT sequence that codes for glycine ("Gly"). A small percentage of the cells contain the G to A mutation in GAT, which codes for aspartic acid ("Asp"). As illustrated, this process involves an initial PCR amplification in step 1, an LDR procedure in step 2, and a separation of fluorescent products followed by quantification in step 3. The LDR probes for wild-type (i.e. normal) sequences are used at low level and/or are modified to yield

less ligation product sequence corresponding to wild type target nucleotide sequence. As shown in FIG. 6, the existence of a 44 base ligation product sequence with fluorescent label F1 coupled to a single nucleotide (designated N.sub.1) and the An tail indicates the presence of the aspartic acid encoding mutant. This ligation product sequence has the same F1 label as that formed by the existence of the arginine and valine encoded mutations. However, these sequences are distinguishable by virtue of their different lengths due to different length tails and different numbers of nucleotides N coupling the label to the remainder of the ligation product sequence. More particularly, the presence of an F1 labelled, 48 base ligation product sequence suggests the presence of the arginine encoding codon, while the presence of an F1 labelled, 46 base ligation product sequence indicates the presence of the valine encoding codon. These ligation product sequences are distinguished by size with the longer products having a lower electrophoretic mobility. The F2 labelled ligation products are similarly distinguished by their length which varies as a result of the different length tails and the number of nucleotides N coupling the label to the remainder of the ligation product sequence. More particularly, the 49 base ligation product sequence (due to 2 nucleotides N coupling the label and the A.sub.n+4 tail) indicates the presence of the cysteine encoding codon, the 47 base ligation product sequence (due to no nucleotides N coupling the label and the A.sub.n+4 tail) indicates the presence of the serine encoding codon, and the 45 base ligation product sequence (due to 2 nucleotides N coupling the label and the A.sub.n tail) indicates the presence of the alanine encoding codon. The ligation product formed by the wild type allele-specific oligonucleotide probe is 43 bases and has the F.sub.2 label (due to 0 nucleotides N coupling the label and the A.sub.n tail). In the labelled probe forming that ligation product, there is a base N located 3 base positions away from the ligation junction which can be either the conventional, proper nucleotide for the wild type target (if that probe is used at low level), or a mismatch, or a nucleotide base analogue. Use of a mismatched nucleotide, a nucleotide base analogue, and/or a modification in the sugar phosphate backbone reduces the amount of ligation product formed off wild-type target. Thus, the presence of wild type target can be detected without overwhelming the signal generated by the presence of minority mutant target. The amount of minority target nucleotide sequences in the sample is determined by comparing the amount of low yield ligation product sequences generated from the majority target nucleotide sequences with the amount of other ligation products.

Detailed Description Text (43):
FIGS. 4-6 show the use of the ligase detection reaction to detect mismatches at the 3' end of the distinguishing oligonucleotide probe. In other cases, however, the mismatch can be at the penultimate position to the 3' end or and the third position away from the 3' end.

Detailed Description Text (45):
FIG. 7 is a schematic diagram depicting the PCR/LDR process of FIG. 1 for detection of cancer-associated mutations at adjacent alleles using an addressable array. FIG. 7 relates to the detection of codon 12 of the K-ras gene which has a wild-type GGT sequence that codes for glycine ("Gly") and minority mutant GAT sequence coding for aspartic acid ("Asp"). The process of FIG. 7 involves an initial PCR amplification in step 1, an LDR procedure in step 2, and capture on a solid support in step 3. As in FIG. 4, the LDR probes for the wild-type target sequence are missing from the reaction to avoid overwhelming signal produced by the mutant target sequence. According to this embodiment of the present invention, as shown in FIG. 7, the presence of the aspartic acid encoding GGT sequence produces a ligation product sequence with label F and addressable array-specific portion Z4. The existence of such a ligation product sequence is indicated by the presence of a nucleic acid, having label F, hybridized at an address on a solid support with a capture oligonucleotide complementary to addressable array-specific portion Z4. As shown in FIG. 7, the support has an array of addresses with capture oligonucleotides complementary to different addressable array-specific portions Z1 to Z6. Since common oligonucleotide probes with label F are used, by observing which site on the solid support they hybridize to, different ligation product sequences are distinguished.

Detailed Description Text (46):
FIG. 8 is a schematic diagram depicting the PCR/LDR process of FIG. 2 for detection of cancer-associated mutations at adjacent alleles using an addressable array. FIG. 8 relates to the detection of codon 12 of the K-ras gene which has a wild-type GGT

sequence that codes for glycine ("Gly") and minority mutant GAT sequence coding for aspartic acid ("Asp"). The process of FIG. 8 involves an inital PCR amplification in step 1, an LDR procedure in step 2, and capture on a solid support in step 3. As in FIG. 5, the LDR probes for the wild-type target sequence are missing from the reaction to avoid overwhelming signal produced by the mutant target sequence. According to this embodiment of the present invention, as shown in FIG. 8, the presence of the aspartic acid encoding AT sequence produces a ligation product sequence with label F and addressable array-specific portion Z4. The existence of such a ligation product sequence is indicated by the presence of a nucleic acid, having label F, hybridized at an address on a solid support with a capture oligonucleotide complementary to addressable array-specific portion Z4. As shown in FIG. 8, the support has an array of addresses with capture oligonucleotides complementary to different addressable array-specific portions Z1 to Z7. Since common oligonucleotide probes with label F are used, by observing which site on the solid support they hybridize to, different ligation product sequences are distinguished. The presence of ligation product sequence produced from a marker-specific probe is indicated by the existence of a nucleic acid, having label F, hybridized at an address on a solid support with a capture oligonucleotide complementary to addressable array-specific portion Z7. The amount of target nucleotide sequences in the sample is determined by comparing the amount of ligation product sequence generated from known amounts of marker target nucleotide sequences with the amount of other ligation product sequences.

Detailed Description Text (47):
FIG. 9 is a schematic diagram depicting the PCR/LDR process of FIG. 3 for detection of cancer-associated mutations at adjacent alleles using an addressable array. FIG. 9 relates to the detection of codon 12 of the K-ras gene which has a wild-type GGT sequence that codes for glycine ("Gly") and minority mutant GAT sequence coding for aspartic acid ("Asp"). The process of FIG. 9 involves an inital PCR amplification in step 1, an LDR procedure in step 2, and capture on a solid support in step 3. The LDR probes for wild-type (i.e. normal) sequences are used at low level and/or are modified to yield less ligation product sequence corresponding to wild type target nucleotide sequence. According to this embodiment of the present invention, as shown in FIG. 9, the presence of the aspartic acid encoding GAT sequence produces a ligation product sequence with label F and addressable array-specific portion Z4. The existence of such a ligation product sequence is indicated by the presence of a nucleic acid, having label F, hybridized at an address on a solid support with a capture oligonucleotide complementary to addressable array-specific portion Z4. As shown in FIG. 9, the support has an array of addresses with capture oligonucleotides complementary to different addressable array-specific portions Z1 to Z7. Since common oligonucleotide probes with label F are used, different ligation product sequences are distinguished by which site on the solid support they hybridize to. The ligation product formed by the wild type allele-specific oligonucleotide probe is indicated by the existence of a nucleic acid, having label F, hybridized at an address on a solid support with a capture oligonucleotide complementary to addressable array-specific portion Z7. In the labelled probe forming that ligation product, there is a base N located 3 base positions away from the ligation junction that can be either a conventional nucleotide for the wild type target (if that probe is used at low level), or a mismatch nucleotide, or a nucleotide base analogue. Use of a mismatched nucleotide, a nucleotide base analogue, and/or a modification in the sugar phosphate backbone reduces the amount of ligation product formed off wild-type target. Thus, the presence of wild type target can be detected without overwhelming the signal generated by the presence of minority mutant target. The amount of minority target nucleotide sequences in the sample is determined by comparing the amount of low yield ligation product sequences generated from the majority target nucleotide sequences with the amount of other ligation products.

Detailed Description Text (56):
The use of a mutant ligase in accordance with the process of the present invention can be explained as follows. The specificity of an enzymatic reaction is determined by the catalytic constant, k.sub.cat, and the apparent binding constant, K.sub.M, and expressed as the specificity constant k.sub.cat /K.sub.M. Any modifications made on the enzyme itself, substrate, or reaction conditions, which affect k.sub.cat or K.sub.M or both, will change the specificity. The use of a mutant enzyme may influence the stability of the perfect matched and mismatched enzyme-DNA complexes to a different extent, so that discrete K.sub.M effects are exerted on these ligation

reactions. In a competitive reaction, such as ligation of perfectly matched and mismatched substrates, the ratio of the specificity constant may be altered as a consequence of K.sub.M, and possible k.sub.cat changes for each substrate. All mutant enzymes which satisfy the equation below (shown for K294R) will give increased discrimination of cancer-associated mutations in the presence of an excess of normal DNA. ##EQU1##

Detailed Description Text (112):
A KTDG motif was deduced to be the active site of the mRNA capping enzymes of the Vaccinia virus (Cong, P., et al., J. Biol. Chem., 268:7256-60 (1993), which is hereby incorporated by reference), S. cerevisiae (Fresco, L. D., et al., Proc. Natl. Acad. Sci. USA, 91:6624-28 (1994) and Schwer, B., et al., Proc. Natl. Acad. Sci. USA, 91:4328-32 (1994), which are hereby incorporated by reference), and S. pombe (Shuman, S., et al., Proc. Natl. Acad. Sci. USA, 91:12046-50 (1994), which is hereby incorporated by reference), for enzyme-guanylate formation. In Yeast tRNA ligase, the amino acid sequence KANG was identified by sequencing the adenylated peptide (Xu, Q., et al., Biochemistry, 29:6132-38 (1990), which is hereby incorporated by reference). A comparison of 5 capping enzymes and 14 ATP dependent DNA and RNA ligases suggests a superfamily of five evolutionarily conserved motifs which plays a role in nucleotidyl binding and transfer to an RNA or DNA substrate (Shuman, S., et al., Molec. Microbiol., 17:405-10 (1995); Shuman, S., et al., Proc. Natl. Acad. Sci. USA, 91:12046-50 (1994); and Cong, P., et al., Molec. Cell. Biol., 15:6222-31 (1995), which are hereby incorporated by reference). These earlier studies, plus the present work on an NAD.sup.+ requiring ligase, allow us to consider KXDING as a general active site motif for creating a charged enzyme-nucleotide complex, which provides the energy to form a covalent phosphodiester bond in nucleic acid substrates.

Detailed Description Text (113):
The observation that the double mutant (K294P/G339E) lost ligase activity suggests that G339 may be important for the third step of the ligation reaction; i.e. formation of the phosphodiester bond. To confirm that this effect is caused by one mutation at G339 site, and not by an additive effect of two mutations, single amino acid substitutions were made at G339 by site-directed mutagenesis. Site-specific mutations were also made at R337, a conserved positively charged amino acid near G339, and at C412, C415, C428, and C433 (FIG. 11). There are only four Cys residues in Tth DNA ligase, all conserved among the five NAD.sup.+ -dependent bacterial ligases that are sequenced. These four Cys residues may form a zinc-binding site and be involved in the interaction between bacterial DNA ligase and DNA substrates (Thorbjarnardottir, S. H., et al., Gene, 161:1-6 (1995), which is hereby incorporated by reference).

Detailed Description Text (120):
Oligonucleotide probes were synthesized using reagents and a model 394 automated DNA synthesizer from Applied Biosystems Division of Perkin-Elmer Corporation, (Foster City, Calif.). Fluorescent label was attached to the 5' end of oligonucleotides using 6-FAM (6-carboxy fluorescein) amidites, or attached to a 3'-amino group (C3-CPG column from Glen Research (Sterling, Va.)) using NHS-FAM (N-hydroxysuccinimide ester of FAM) from Applied Biosystems Division of Perkin-Elmer Corporation. A universal nucleotide analogue, 1-(2'-deoxy-.beta.-D-ribofuranosyl)-3-nitropyrrole, herein designated as Q, was synthesized, transformed to the phosphoramidite, and oligonucleotides synthesized as described (Bergstrom, D. E., et al., JACS, 117:1201-1209 (1995), which is hereby incorporated by reference). All oligonucleotides used in this study were purified by polyacrylamide gel electrophoresis with recovery of DNA from gel slices using C-18 Sep-Pak Cartridges from Waters Division of Millipore (Bedford, Mass.).

Detailed Description Text (139):
Sequences of these probes (shown in FIG. 14) were derived from that of human eukaryotic protein synthesis initiation factor eIF-4E (Rychlik, W., et al., "Amino Acid Sequence of the mRNA Cap-Binding Protein From Human Tissue," Proc. Natl. Acad. Sci. USA, 84:945-949 (1987), which is hereby incorporated by reference). A random DNA sequence from a eukaryotic source was chosen to avoid any false signal arising from possible bacterial DNA contamination in Tth DNA ligase preparation. The melting temperature of probes were predicted using the nearest neighbor thermodynamic method (Breslauer, K. J., et al., "Predicting DNA Duplex Stability From the Base Sequence," Proc. Natl. Acad. Sci. USA, 83:3746-3750 (1986), which is hereby incorporated by reference). OLIGO 4.0 program from National Biosciences Inc., Plymouth, Minn. was used

to rule out possible hairpin structure, repetitive sequences, and false priming. The templates and detecting oligonucleotides for this assay have been designed such that their melting temperature is sufficiently higher than the temperature used in the assay (65.degree. C.) to minimize the effect of the melting temperature of probes on ligation efficiency.

Detailed Description Text (158):
An elegant method for improving allele-specific PCR is based on using primers with a deliberate mismatch adjacent to the discriminating 3' base (Cha, R. S., et al., PCR Methods and Applications, 2:14-20 (1992) and Rust, S., et al., Nucleic Acids Res., 21(16): 3623-3629 (1993), which are hereby incorporated by reference). This destabilizing mismatch did not dramatically reduce Taq polymerase extension of the correct target allele, but owing to a double mismatch of the other allele, the extension efficiency of the incorrect allele was reduced by a factor of I 00 to 1000-fold (Cha, R. S., et al., PCR Methods and Applications, 2:14-20 (1992), which is hereby incorporated by reference).

Detailed Description Text (163):
The fidelity of DNA polymerases may be decreased by site-directed mutagenesis of motifs associated with primer-template binding (HIV polymerase) (Beard, W. A., et al., J. Biol. Chem., 269:28091-28097 (1994), which is hereby incorporated by reference) or the exoIII motif of Phi29 DNA polymerase (Soengas, M. D., et al., The EMBO J., 11(11): 4227-4237 (1992), which is hereby incorporated by reference), T4 DNA polymerase (Reha-Krantz, L. J., et al., J. Virol., 67(1): 60-66 (1993) and Reha-Krantz, L. J., et al., J. Biol. Chem., 269:5635-5643 (1994), which are hereby incorporated by reference), or human DNA polymerase alpha (Dong, Q., et al., J. Biol. Chem., 268:24163-24174 (1993), which is hereby incorporated by reference). Occasionally, this same exolIl motif or motif "A" yields increased fidelity mutants also known as "antimutator" strains, which reflects the complex interplay between the polymerizing and 3'-5' exonuclease activities of these enzymes in modulating overall fidelity (Reha-Krantz, L. J., et al., J. Virol., 67(1): 60-66 (1993); Reha-Krantz, L. J., et al., J. Biol. Chem., 269:5635-5643 (1994); Dong, Q., et al., J. Biol. Chem., 268:24175-24182 (1993); and Copeland, W. C., et al., J. Biol. Chem., 268:11041-11049 (1993), which are hereby incorporated by reference).

Detailed Description Text (179):
(The K294P mutation causes loss of thermostability and, therefore, was not used in further studies). The amount of LDR product generated (0.56 fmol) by the mutant K294R on the mismatched template, was reduced by more than 2-fold as compared to the wild-type enzyme (1.45 fmol). LDR was also performed with the perfect match and mismatch templates together in a ratio of 1:25 and 1:100, respectively. For both wild-type and mutant enzymes, the product generated in the presence of the mismatch template was less than the product generated by the perfect match template alone. The use of K294R results in a 1.75 to 3 fold higher signal-to-noise ratio. Since no background was detected with the K294L mutant, a signal-to-noise ratio could not be calculated. Thus, these results support the finding that the mutant K294R exhibits a higher fidelity in discriminating a mismatch over the wild-type enzyme. This increased fidelity is probably due to a change in the specificity constant of this mutant thermostable enzyme. The specificity of an enzymatic reaction is determined by the catalytic constant, k.sub.cat, and the apparent binding constant, K.sub.M, and expressed as the specificity constant k.sub.cat /K.sub.M. Any modifications made on the enzyme itself, substrate, or reaction conditions, which affect k.sub.cat or K.sub.M or both, will change the specificity. The use of a mutant enzyme may influence the stability of the perfect matched and mismatched enzyme-DNA complexes to a different extent, so that discrete K.sub.M effects are exerted on these ligation reactions. In a competitive reaction, such as ligation of perfectly matched and mismatched substrates, the ratio of the specificity constant may be altered as a consequence of K.sub.M, and possible k.sub.cat changes for each substrate. All mutant enzymes which satisfy the equation below (shown for K294R) will give increased discrimination of cancer-associated mutations in the presence of an excess of normal DNA. ##EQU12##

CLAIMS:

8. A method according to claim 5, wherein the oligonucleotide probe sets form a

plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein, in the oligonucleotide probe sets of each group, there is a common first oligonucleotide probe, having a detectable reporter label, and a second oligonucleotide probe which hybridizes to a given allele or marker nucleotide sequence in a base-specific manner, each second oligonucleotide probe having a different length and, wherein the ligation product sequences of oligonucleotide probes in a particular set generate a unique length product, said method further comprising:

separating the ligation product sequences by size or electrophoretic mobility and

distinguishing the ligation product sequences which differ in size.

9. A method according to claim 5, wherein the oligonucleotide probe sets form a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein, in the oligonucleotide probe sets of each group, there is a common first oligonucleotide probe and a second oligonucleotide probe which hybridizes to a given allele or marker nucleotide sequence in a base-specific manner, each second oligonucleotide probe having a different detectable reporter label and wherein the ligation product sequences of oligonucleotide probes in a particular set generate a unique length product, said method further comprising:

separating the ligation product sequences by size or electrophoretic mobility and

distinguishing the ligation product sequences which differ in size.

10. A method according to claim 6, wherein the oligonucleotide probe sets form a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein, in the oligonucleotide probe sets of each group, there is a common first oligonucleotide probe having a detectable reporter label and a second oligonucleotide probe which hybridizes to a given allele or a marker nucleotide sequence in a base-specific manner, each second oligonucleotide probe each having different addressable array-specific portions.

11. A method according to claim 6, wherein the oligonucleotide probe sets form a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein, in the oligonucleotide probe set of each group, there is a common first oligonucleotide probe, having an addressable array-specific portion, and a second oligonucleotide probe which hybridizes to a given allele or a marker nucleotide sequence in a base-specific manner, each second oligonucleotide probe having a different detectable reporter label.

13. A method according to claim 1, wherein a low abundance of multiple allele differences at multiple nearby or adjacent positions in a single target nucleotide sequence in the presence of an excess of normal sequence, or a low abundance of multiple allele differences at multiple nearby positions in multiple target nucleotide sequences in the presence of an excess of normal sequence in a sample are distinguished, the oligonucleotide probe set forming a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein one or more sets within a group share common first oligonucleotide probes, and the second oligonucleotide probes hybridize to a given allele excluding the normal allele in a base-specific manner, wherein, in said detecting, the labels of ligation product sequences are detected, thereby indicating a presence, in the sample, of one or more low abundance alleles at one or more nucleotide positions in one or more target nucleotide sequences.

14. A method according to claim 1, wherein a low abundance of multiple allele differences at multiple nearby or adjacent positions in a single target nucleotide sequence, in the presence of an excess of normal sequence, or a low abundance of multiple allele differences at multiple nearby positions in multiple target nucleotide

sequences, in the presence of an excess of normal sequence, in the sample in unknown amounts are quantified, said method further comprising:

providing a known amount of one or more marker target nucleotide sequences;

providing one or more marker-specific oligonucleotide probe sets, each set characterized by (a) a first oligonucleotide probe having a target-specific portion complementary to a marker target nucleotide sequence and (b) a second oligonucleotide probe, having a target-specific portion complementary to a marker target nucleotide sequence and a detectable reporter label, wherein the oligonucleotide probes in a particular marker-specific oligonucleotide set are suitable for ligation together when hybridized adjacent to one another on a corresponding marker target nucleotide sequence, but, when hybridized to any other nucleotide sequence present in the sample or added marker sequences, have a mismatch which interferes with such ligation, wherein said plurality of oligonucleotide probe sets and said plurality of marker-specific oligonucleotide probe sets may form oligonucleotide probe groups for distinguishing multiple allele differences at a single nucleotide position, including marker nucleotide sequences, wherein one or more sets within a group, containing marker-specific oligonucleotide probes, share a common first oligonucleotide probe and a second oligonucleotide probe which hybridizes to a given allele excluding the normal allele in a base-specific manner, wherein said blending to form the ligase detection reaction mixture comprises blending the marker target nucleotide sequences and the probe sets specifically designed for the marker target nucleotide sequences with the ligase detection reaction mixture;

quantifying the amount of ligation product sequences; and

comparing the amount of ligation product sequences generated from the low abundance unknown sample with the amount of ligation product sequences generated from known amounts of marker target nucleotide sequences, to provide a quantitative measure of the level of one or more low abundance target nucleotide sequences in the sample.

15. A method according to claim 1, wherein a low abundance of multiple allele differences at multiple nearby or adjacent positions in a single target nucleotide sequence in the presence of an excess of normal sequence, or a low abundance of multiple allele differences at multiple nearby positions in multiple target nucleotide sequences, in the presence of an excess of normal sequence in a sample, are distinguished, the oligonucleotide probe sets forming a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein one or more sets within a group share common first oligonucleotide probes, and the second oligonucleotide probes hybridize to a given allele excluding the normal allele in a base-specific manner, wherein one or more sets within a group share common first oligonucleotide probes, and the second oligonucleotide probes hybridize to a given allele including the normal allele in a base-specific manner, wherein the normal allele-specific probes are present in the ligation detection reaction mixture at a lower concentration than the mutant allele-specific probes, whereby the quantity of ligation product generated from the majority normal target is similar to the quantity of ligation product sequence expected from a known dilution of the minority target,

quantifying the amount of ligation product sequences;

comparing the amount of ligation product sequences generated from the low abundance unknown sample with the amount of ligation product sequences generated from the high abundance normal target nucleotide sequences; and

determining the amount of the high abundance normal target nucleotide sequences based on the low level of normal ligation product sequence formation, to provide a quantitative measure of the ratio of one or more low abundance target nucleotide sequences compared to the the high abundance normal target nucleotide sequences in the sample.

16. A method according to claim 1, wherein a low abundance of multiple allele differences at multiple nearby or adjacent positions in a single target nucleotide sequence in the presence of an excess of normal sequence, or a low abundance of

multiple allele differences at multiple nearby positions in multiple target nucleotide sequences, in the presence of an excess of normal sequence in a sample, are distinguished, the oligonucleotide probe sets forming a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein one or more sets within a group share common first oligonucleotide probes, and the second oligonucleotide probes hybridize to a given allele excluding the normal allele in a base-specific manner, wherein one or more sets within a group share common first oligonucleotide probes, and the second oligonucleotide probes hybridize to a given allele including the normal allele in a base-specific manner, wherein the normal allele-specific probes are present in the ligation detection reaction mixture at a lower concentration and/or contain one or more internal mismatches or modifications, whereby the quantity of ligation product sequence generated from the majority normal target is similar to the quantity of ligation product sequence expected from a known dilution of the minority target,

quantifying the amount of ligation product sequences;

comparing the amount of ligation product sequences generated from the low abundance unknown sample with the amount of ligation product sequences generated from the high abundance normal target nucleotide sequences; and

determining the amount of the high abundance normal target nucleotide sequences based on the low level of normal ligation product sequence formation, to provide a quantitative measure of the ratio of one or more low abundance target nucleotide sequences compared to the the high abundance normal target nucleotide sequences in the sample.

17. A method according to claim 16, wherein the normal allele-specific probes contain the normal base at the 3' end.

18. A method according to claim 17, wherein the normal allele-specific probes contain the one or more internal mismatches or modifications within 4 bases from the 3' end.

33. A method according to claim 30, wherein the oligonucleotide probe sets form a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein, in the oligonucleotide probe sets of each group, there is a common first oligonucleotide probe, having detectable reporter label, and a second oligonucleotide probe which hybridizes to a given allele or marker nucleotide sequence in a base-specific manner, each second oligonucleotide probe having a different length and, wherein the ligation product sequences of oligonucleotide probes in a particular set generate a unique length product, said method further comprising:

separating the ligation product sequences by size or electrophoretic mobility and

distinguishing the ligation product sequences which differ in size.

34. A method according to claim 30, wherein the oligonucleotide probe sets form a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein, in the oligonucleotide probe sets of each group, there is a common first oligonucleotide probe and a second oligonucleotide probe which hybridizes to a given allele or marker nucleotide sequence in a base-specific manner, each second oligonucleotide probe having a different detectable reporter label and wherein the ligation product sequences of oligonucleotide probes in a particular set generate a unique length product, said method further comprising:

separating the ligation product sequences by size or electrophoretic mobility and

distinguishing the ligation product sequences which differ in size.

35. A method according to claim 31, wherein the oligonucleotide probe sets form a plurality of oligonucleotide probe groups, each group comprised of one or more

oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein, in the oligonucleotide probe sets of each group, there is a common first oligonucleotide probe having a detectable reporter label and a second oligonucleotide probe which hybridizes to a given allele or a marker nucleotide sequence in a base-specific manner, each second oligonucleotide probe having different addressable array-specific portions.

36. A method according to claim 31, wherein the oligonucleotide probe sets form a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein, in the oligonucleotide probe set of each group, there is a common first oligonucleotide probe, having an addressable array-specific portion, and a second oligonucleotide probe which hybridizes to a given allele or a marker nucleotide sequence in a base-specific manner, each second oligonucleotide probe having a different detectable reporter label.

38. A method according to claim 25, wherein a low abundance of multiple allele differences at multiple nearby or adjacent positions in a single target nucleotide sequence in the presence of an excess of normal sequence, or a low abundance of multiple allele differences at multiple nearby positions in multiple target nucleotide sequences in the presence of an excess of normal sequence in a sample are distinguished, the oligonucleotide probe set forming a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein one or more sets within a group share common first oligonucleotide probes, and the second oligonucleotide probes hybridize to a given allele excluding the normal allele in a base-specific manner, wherein, in said detecting, the labels of ligation product sequences are detected, thereby indicating a presence, in the sample, of one or more low abundance alleles at one or more nucleotide positions in one or more target nucleotide sequences.

39. A method according to claim 25, wherein a low abundance of multiple allele differences at multiple nearby or adjacent positions in a single target nucleotide sequence, in the presence of an excess of normal sequence, or a low abundance of multiple allele differences at multiple nearby positions in multiple target nucleotide sequences, in the presence of an excess of normal sequence, in the sample in unknown amounts are quantified, said method further comprising:

providing a known amount of one or more marker target nucleotide sequences;

providing one or more marker-specific oligonucleotide probe sets, each set characterized by (a) a first oligonucleotide probe having a target-specific portion complementary to a marker target nucleotide sequence and (b) a second oligonucleotide probe, having a target-specific portion complementary to a marker target nucleotide sequence and a detectable reporter label, wherein the oligonucleotide probes in a particular marker-specific oligonucleotide set are suitable for ligation together when hybridized adjacent to one another on a corresponding marker target nucleotide sequence, but, when hybridized to any other nucleotide sequence present in the sample or added marker sequences, have a mismatch which interferes with such ligation, wherein said plurality of oligonucleotide probe sets and said plurality of marker-specific oligonucleotide probe sets may form oligonucleotide probe groups for distinguishing multiple allele differences at a single nucleotide position, including marker nucleotide sequences, wherein one or more sets within a group, containing marker-specific oligonucleotide probes, share a common first olig nucleotide probe and a second oligonucleotide probe which hybridizes to a given allele excluding the normal allele in a base-specific manner, wherein said blending to form the ligase detection reaction mixture comprises blending the marker target nucleotide sequences and the probe sets specifically designed for the marker target nucleotide sequences with the ligase detection reaction mixture;

quantifying the amount of ligation product sequences; and

comparing the amount of ligation product sequences generated from the low abundance unknown sample with the amount of ligation product sequences generated from known amounts of marker target nucleotide sequences, to provide a quantitative measure of

the level of one or more low abundance target nucleotide sequences in the sample.

40. A method according to claim 25, wherein low abundance of multiple allele differences at multiple nearby or adjacent positions in a single target nucleotide sequence in the presence of an excess of normal sequence, or a low abundance of multiple allele differences at multiple nearby positions in multiple target nucleotide sequences, in the presence of an excess of normal sequence in a sample, are distinguished, the oligonucleotide probe sets forming a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein one or more sets within a group, share common first oligonucleotide probes, and the second oligonucleotide probes, hybridize to a given allele excluding the normal allele in a base-specific manner, wherein one or more sets within a group share common first oligonucleotide probes, and the second oligonucleotide probes hybridize to a given allele including the normal allele in a base-specific manner, wherein the normal allele-specific probes are present in the ligation detection reaction mixture at a lower concentration than he mutant allele-specific probes, whereby the quantity of ligation product sequence generated from the majority normal target is similar to the quantity of ligation product sequence expected from a known dilution of the minority target,

quantifying the amount of ligation product sequences;

comparing the amount of ligation product sequences generated from the low abundance unknown sample with the amount of ligation product sequences generated from the high abundance normal target nucleotide sequences; and

determining the amount of the high abundance normal target nucleotide sequences based on the low level of normal ligation product sequence formation, to provide a quantitative measure of the ratio of one or more low abundance target nucleotide sequences compared to the the high abundance normal target nucleotide sequences in the sample.

41. A method according to claim 25, wherein, low abundance of multiple allele differences at multiple nearby or adjacent positions in a single target nucleotide sequence in the presence of an excess of normal sequence, or a low abundance of multiple allele differences at multiple nearby positions in multiple target nucleotide sequences, in the presence of an excess of normal sequence in a sample, are distinguished, the oligonucleotide probe set forming plurality of oligonucleotide probe groups, each group comprised of one or ore oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein one or more sets within a group share common first oligonucleotide probes, and the second oligonucleotide probes hybridize to a given allele excluding the normal allele in a base-specific manner, wherein one or more sets within a group share common first oligonucleotide probes, and the second oligonucleotide probes hybridize to a given allele including the normal allele in a base-specific manner, wherein the normal allele-specific probes are present in the ligation detection reaction mixture at a lower concentration and/or contain one or more internal mismatches or modifications, whereby the quantity of ligation product sequence generated from the majority normal target is similar to the quantity of ligation product sequence expected from a known dilution of the minority target,

quantifying the amount of ligation product sequences;

comparing the amount of ligation product sequences generated from the low abundance unknown sample with the amount of ligation product sequences generated from the high abundance normal target nucleotide sequences;

determining the amount of the high abundance normal target nucleotide sequences based on the low level of normal ligation product sequence formation, to provide a quantitative measure of the ratio of one or more low abundance target nucleotide sequences compared to the the high abundance normal target nucleotide sequences in the sample.

42. A method according to claim 41, wherein the normal allele-specific probes contain

the normal base at the 3' end.

43. A method according to claim 42, wherein the normal allele-specific probes contain the one or more internal mismatches or modifications within 4 bases from the 3' end.

63. A method according to claim 60, wherein the oligonucleotide probe sets form a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein, in the oligonucleotide probe sets of each group, there is a common first oligonucleotide probe, having a a detectable reporter label, and a second oligonucleotide probe which hybridizes to a given allele or marker nucleotide sequence in a base-specific manner, each second oligonucleotide probe having a different length and, wherein the ligation product sequences of oligonucleotide probes in a particular set generate a unique length product, said method further comprising:

separating the ligation product sequences by size or electrophoretic mobility and

distinguishing the ligation product sequences which differ in size.

64. A method according to claim 60, wherein the oligonucleotide probe sets form a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein, in the oligonucleotide probe sets of each group, there is a common first oligonucleotide probe and a second oligonucleotide probe which hybridizes to a given allele or marker nucleotide sequence in a base-specific manner, each second oligonucleotide probe having a different detectable reporter label and wherein the ligation product sequences of oligonucleotide probes in a particular set generate a unique length product, said method further comprising:

separating the ligation product sequences by size or electrophoretic mobility and

distinguishing the ligation product sequences which differ in size.

65. A method according to claim 61, wherein the oligonucleotide probe sets form a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein, in the oligonucleotide probe sets of each group, there is a common first oligonucleotide probe having a detectable reporter label and a second oligonucleotide probe which hybridizes to a given allele or a marker nucleotide sequence in a base-specific manner, each second oligonucleotide probe having different addressable array-specific portions.

66. A method according to claim 61, wherein the oligonucleotide probe sets form a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein, in the oligonucleotide probe set of each group, there is a common first oligonucleotide probe, having an addressable array-specific portion, and a second oligonucleotide probe which hybridizes to a given allele or a marker nucleotide sequence in a base-specific manner, each second oligonucleotide probe having a different detectable reporter label.

68. A method according to claim 50, wherein a Low abundance of multiple allele differences at multiple nearby or adjacent positions in a single target nucleotide sequence in the presence of an excess of normal sequence, or a low abundance of multiple allele differences at multiple nearby positions in multiple target nucleotide sequences in the presence of an excess of normal sequence in a sample are distinguished, the oligonucleotide probe set forming a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein one or more sets within a group share common first oligonucleotide probes, and the second oligonucleotide probes hybridize to a given allele excluding the normal allele in a base-specific manner, wherein, in said detecting, the labels of ligation product sequences are detected, thereby indicating a presence, in the sample, of one or more low abundance alleles at one or more nucleotide positions in one or more

target nucleotide sequences.

69. A method according to claim 50, wherein a low abundance of multiple allele differences at multiple nearby or adjacent positions in a single target nucleotide sequence, in the presence of an excess of normal sequence, or a low abundance of multiple allele differences at multiple nearby positions in multiple target nucleotide sequences, in the presence of an excess of normal sequence, in the sample in unknown amounts are quantified, said method further comprising:

providing a known amount of one or more marker target nucleotide sequences;

providing one or more marker-specific oligonucleotide probe sets, each set characterized by (a) a first oligonucleotide probe having a target-specific portion complementary to a marker target nucleotide sequence and (b) a second oligonucleotide probe, having a target-specific portion complementary to a marker target nucleotide sequence and a detectable reporter label, wherein the oligonucleotide probes in a particular marker-specific oligonucleotide set are suitable for ligation together when hybridized adjacent to one another on a corresponding marker target nucleotide sequence, but, when hybridized to any other nucleotide sequence present in the sample or added marker sequences, have a mismatch which interferes with such ligation, wherein said plurality of oligonucleotide probe sets and said plurality of marker-specific oligonucleotide probe sets may form oligonucleotide probe groups for distinguishing multiple allele differences at a single nucleotide position, including marker nucleotide sequences, wherein one or more sets within a group, containing marker-specific oligonucleotide probes, share a common first oligonucleotide probe and a second oligonucleotide probe which hybridizes to a given allele excluding the normal allele in a base-specific manner, wherein said blending to form the ligase detection reaction mixture comprises blending the marker target nucleotide sequences and the probe sets specifically designed for the marker target nucleotide sequences with the ligase detection reaction mixture;

quantifying the amount of ligation product sequences; and

comparing the amount of ligation product sequences generated from the low abundance unknown sample with the amount of ligation product sequences generated from known amounts of marker target nucleotide sequences, to provide a quantitative measure of the level of one or more low abundance target nucleotide sequences in the sample.

70. A method according to claim 50, wherein a low abundance of multiple allele differences at multiple nearby or adjacent positions in a single target nucleotide sequence in the presence of an excess of normal sequence, or a low abundance of multiple allele differences at multiple nearby positions in multiple target nucleotide sequences, in the presence of an excess of normal sequence in a sample, are distinguished, the oligonucleotide probe sets forming a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein one or more sets within a group shire common first oligonucleotide probes, and the second oligonucleotide probes hybridize to a given allele excluding the normal allele in a base-specific manner, wherein one or more sets within a group share common first oligonucleotide probes, and the second oligonucleotide probes hybridize to a given allele including the normal allele in a base-specific manner, wherein the normal allele-specific probes are present in the ligation detection reaction mixture at a lower concentration than the mutant allele-specific probes, whereby the quantity of ligation product sequence generated from the majority normal target is similar to the quantity of ligation product sequence expected from a known dilution of the minority target;

quantifying the amount of ligation product sequences;

comparing the amount of ligation product sequences generated from the low abundance unknown sample with the amount of ligation product sequences generated from the high abundance normal target nucleotide sequences; and

determining the amount of the high abundance normal target nucleotide sequences based on the low level of normal ligation product sequence formation, to provide a

quantitative measure of the ratio of one or more low abundance target nucleotide sequences compared to the the high abundance normal target nucleotide sequences in the sample.

71. A method according to claim 50, wherein a low abundance of multiple allele differences at multiple nearby or adjacent positions in a single target nucleotide sequence in the presence of an excess of normal sequence, or a low abundance of multiple allele differences at multiple nearby positions in multiple target nucleotide sequences, in the presence of an excess of noel sequence in a sample, are distinguished, the oligonucleotide probe sets forming a plurality of oligonucleotide probe groups, each group comprised of one or mere oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, wherein one or more sets within a group share common first oligonucleotide probes, and the second oligonucleotide probes hybridize to a given allele excluding the normal allele in a base-specific manner, wherein one or more sets within a group share common first oligonucleotide probes, and the second oligonucleotide probes hybridize to a given allele including the normal allele in a base-specific manner, wherein the normal allele-specific probes are present in the ligation detection reaction mixture at a lower concentration and/or contain one or more internal mismatches or modifications, whereby the ligation product sequence generated from the majority normal target is similar to the quantity of ligation product sequence expected from a known dilution of the minority target;

quantifying the amount of ligation product sequences;

comparing the amount of ligation product sequences generated from the low abundance unknown sample with the amount of ligation product sequences generated from the high abundance normal target nucleotide sequences; and

determining the amount of the high abundance normal target nucleotide sequences based on the low level of normal ligation product sequence formation, to provide a quantitative measure of the ratio of one or more low abundance target nucleotide sequences compared to the the high abundance normal target nucleotide sequences in the sample.

72. A method according to claim 71, wherein the normal allele-specific probes contain the normal base at the 3' end.

73. A method according to claim 72, wherein the normal allele-specific probes contain the one or more internal mismatches or modifications within 4 bases from the 3' end.